$I_{Ca,L}$ and on the DHEA-induced inhibition of $I_{Ca,L}$ in A7r5, a cell line derived from fetal rat aorta. Chol was depleted with methyl-beta-cyclodextrin (CD) or loaded by cholesterol bound CD (chol-CD). Whole cell $I_{Ca,L}$ was recorded with 10 mM Ba^{2+} as the charge carriers. T-type Ca^{2+} channel current was inactivated by 50 ms pre-pulse to −40 mV. The maximal density of $I_{Ca,L}$ was decreased by 55% by loading Chol with 30 mM chol-CD, while it was not affected by the depletion of Chol by 10 and 30 mM CD. The depletion of Chol by 30 mM CD remarkably decreased the rate of inactivation of $I_{Ca,L,}$ generating sustained current. It shifted $V_{0.5}$ in the f_{∞}-V curve to a depolarizing direction by 10 mV from control −30 mV, with appearance of 27% non-inactivating component at 0 mV. DHEA (30 micro M) inhibited the maximal amplitude of $I_{Ca,L}$ by ~30% irrespective of the manipulation of Chol. It shifted the $V_{0.5}$ to a hyperpolarizing direction by 6 mV in control but 10 mV in the 30 mM CD treated cells. We conclude that membrane cholesterol is essential for the normal voltage-dependent inactivation of L-type Ca^{2+} channel. DHEA, at pharmacological concentrations, more potently augments the inactivation in the cholesterol depleted cells.

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Autism-Associated Point Mutation in $Ca_V1.3$ Calcium Channels alters their Regulation by Ca^{2+}

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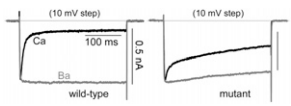
Whole exome sequencing of sporadic autism patients has recently identified *de novo* point mutations absent in healthy siblings (*Nature* (2012) **485**:246). These point mutations thus arise as candidate factors in pathogenesis. Among these mutations is a missense alteration in $Ca_V1.3$ calcium channels, those contributing to rhythmicity throughout the heart and brain. Here, we introduce this missense mutation into the homologous position of rat $Ca_V1.3$ channels, and characterize their functional behavior. Shown on the left are whole-cell currents through wild-type channels, expressed recombinantly in HEK293 cells. The sharp decay of Ca^{2+} (black) versus Ba^{2+} (gray) currents gives evidence of strong Ca^{2+}-dependent inactivation (CDI) of wild-type channels, an important feedback system for Ca^{2+} homeostasis. By contrast, mutant $Ca_V1.3$ channels exhibit sharply diminished CDI (right) and a substantial hyperpolarizing shift in voltage activation. Both features would promote excessive Ca^{2+} influx. This contrast in behavior was maintained across differing splice-variant backgrounds. We speculate that excessive Ca^{2+} entry through $Ca_V1.3$ channels can play an important patho-physiological role in autism.



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A Novel Animal Model to Study the In Vivo Role of a C-Terminal Regulatory Domain in Cav1.3 L-Type Calcium Channels

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Cav1.3 channels-mediated calcium-signals are crucial for hearing, cardiac pacemaking, and for shaping activity patterns in neurons and endocrine cells. We previously found that Cav1.3 activity is strongly modulated by alternative splicing of their pore-forming α1-subunit.α1-variants with a long C-terminus can form a C-terminal modulatory domain (CTM) that reduces open probability, slows inactivation and decreases sensitivity to activation voltage. These modulatory properties are absent in short splice variants, which results in different dynamics of calcium inward current. Long and short splice variants are expressed together in brain and other tissues. However, the (patho-)physiological role of this CTM is unknown.

We therefore generated a mutant mouse strain in which CTM function is disrupted by an HA-tag in one of the putative a-helices (DCRD) forming the CTM. Homozygous mutants (Cav1.3-DCRD-HA/HA mice) are viable and reproduce normally. Heterozygous mice show no overt differences in locomotive activity. As predicted, HA-immunoreactivity in Western blots of mutant mouse brains was only associated with the long Cav1.3 splice variant (230 kDa), and the mutation did not interfere with its protein expression level. Anti-Cav1.3 alpha1-antibodies recognizing all C-terminal splice variants revealed also the presence of short variants (180 kDa). These may arise from alternative splicing and/or from C-terminal post-translational proteolytic processing as described for Cav1.1 and Cav1.2 channels. Proteolytic processing would generate an HA-tagged low molecular mass fragment in Cav1.3-DCRD-HA/HA tissues, a possibility which we currently evaluate. using these animals we will also study the physiological role of CTM function in vivo. Furthermore, the HA-tagged α1-subunit will present an excellent target for specific detection with anti-HA antibodies in mouse tissues.

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Single-Channel Mechanism of Modulation of Calcium-Dependent Inactivation of the Voltage-Gated Calcium Channel Cav1.3 by its C-Terminus

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Background: $Ca_V1.3$ is a L-type calcium channel pore subunit showing calcium-dependent inactivation (CDI). The full-length $Ca_V1.3$ isoform $Ca_V1.3_{42}$ contains a C-terminal modulator (CTM) domain that attenuates calcium-dependent inactivation at the whole-cell current level. $Ca_V1.3$ splice variants lack the CTM domain completely ($Ca_V1.3_{42A}$) or partially ($Ca_V1.3_{43S}$).
Aim of the study: To analyse the role of the CTM domain for single-channel gating of $Ca_V1.3_{42}$, $Ca_V1.3_{42A}$ and $Ca_V1.3_{43S}$ in detail.
Methods: We recorded single-channel calcium and barium currents carried by either $Ca_V1.3_{42}$, $Ca_V1.3_{42A}$ and $Ca_V1.3_{43S}$ plus auxiliary subunits expressed in HEK293 cells. Single-channel CDI was analysed according to Josephson et al. (J Physiol 2010;588:213). By Markov modelling transition rates between channel states were obtained.
Results: As expected single-channel barium currents showed little inactivation during 150ms test pulses. Single-channel activity with barium was different with $Ca_V1.3_{42A}$ being most and $Ca_V1.3_{42}$ least active (Bock et al., J Biol Chem 2011; 286:42736). This difference is due to CTM-dependent changes in the closed-to-open and open-to-closed transition rates across all test potentials examined (−20 to 0 mV). With calcium as charge carrier the $Ca_V1.3$ variants $Ca_V1.3_{42A}$ and $Ca_V1.3_{43S}$ lacking the CTM domain allowed for more pronounced CDI compared to full-length $Ca_V1.3_{42}$. Plotting the ratio of mean open times during late (MOT2) and early (MOT1) period of a depolarizing pulse against cumulative amount of calcium ions passing the channel reveals that a shortening of mean open times underlies CDI of $Ca_V1.3_{42A}$ and $Ca_V1.3_{43S}$. The efficacy of calcium ions to induce this readout parameter of CDI was higher with the isoforms (partly) lacking CTM.
Conclusion: CTM reduces the sensitivity of single $Ca_V1.3$ to CDI. Splice variants affecting the structure of CTM thus will influence the physiological function of e.g. cardiac pacemaker cells or neurons.

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Conserved Asparagine in D4-S6 of Cav1.2 Channel is Critical for Conductance of Divalent Cations

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Current views of selectivity mechanisms in Ca and Na voltage-gated channels focus on the structure made by loops between S5 and S6 segments of the pore-forming subunit. Previous studies, however, also indicated that specifics of S6 segments are important for ion permeability as well.

Here, we examine the role of highly conserved asparagine residue(s) in the middle of otherwise lipophilic S6 segments of all Ca and Na voltage-gated channels. We found that the N1499I mutant at the S6 of the fourth domain of CaV1.2 channels nearly eliminates Ca/Ba currents when expressed in tsA-201 cells. Because the mutation left gating currents unaffected, mutated channels were normally targeted to the plasma membrane. Unexpectedly, the whole-cell currents carried by monovalent ions at sub-micromolar Ca and the single-channel conductance of Li were also unaltered in the N1499I.

To test whether or not the S6-formed physical gate opens in the N1499I at millimolar concentrations of Ca, we introduced an additional mutation E1145K, which dramatically lowers affinity of the selectivity filter for Ca. The N1499I mutation in the S6 had no significant effect on magnitudes of inward Na and outward Cs currents passing at 1 mM Ca through the E1145K selectivity filter mutation.

Our findings show that the N1499I mutant reduces Ca conductance but it does not change activation gating, conductance of monovalent ions, and Ca block of monovalent currents. Therefore, we propose that the mechanism of selective Ca permeation is not structurally confined to the loops between S5 and S6 segments and that other residues in the permeation pathway, such as the conserved asparagine in the S6 of the fourth domain, are also involved.

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